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Is Reliance on Mitochondrial Respiration a "Chink in the Armor" of Therapy-Resistant Cancer?

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A series of recent reports has suggested PGC1α-driven upregulation of mitochondrial oxidative phosphorylation as a selective vulnerability of drug-resistant cancers. Accordingly, chemical inhibitors of respiration led to selective eradication of such cancer cells due to their preferential sensitivity to mitochondrial production of reactive oxygen species. These insights create a timely opportunity for a biomarker guided application of already existing and newly emerging mitochondrial inhibitors in recurrent drug-resistant cancer, including lymphomas, melanomas, and other malignant diseases marked by increased mitochondrial respiration.

Therapy-Selected Quiescence

Current cancer therapeutics have extraordinary efficiency and selectivity, often killing essentially 100% of malignant cells in a culture dish at a dose where normal cells are spared. In vivo, it appears that induction of cell death is never complete. Surviving cells may no longer proliferate, but persist in a quiescent or slow-cycling state for years. This phenotype may have either preexisted in a subset of cells from the heterogeneous tumor mass (e.g., "cancer stem cells") or may have been acquired as a consequence of the cytotoxic treatment. Either way, it is now well established that the very same therapeutic regimen, whether cytotoxic or "targeted," can drive both apoptotic cell death and selection of therapy resistant quiescent or slow-cycling cells, a process I will refer to as therapy-selected quiescence (TSQ). Cells undergoing TSQ may be thought of as "cancer memory cells" that build a reservoir to fuel cancer recurrence (Figure 1A).

A well established form of TSQ is therapy-induced senescence (TIS). Common therapies such as radiation and genotoxic chemotherapy trigger either apoptosis or TIS, with the specific response being determined by the dose. High doses cause cell death, whereas lower doses induce TIS. The heterogeneity of the tumor environment may lead to gradients in drug exposure that enable the coexistence of both therapeutic responses. Senescence is mediated and maintained by cyclin-dependent kinase inhibitors—$p16^{Ink4a}$ (CDKN2A), $p21^{WAF1,CIP1}$ (CDKN1A), and $p27^{KIP1}$ (CDKN1B)—that are induced downstream of tumor suppressor pathways including p53- and RB1-related proteins (Schmitt et al., 2002). However, TIS also occurs in the absence of these tumor suppressor pathways because they are frequently defective in cancer cells that undergo TIS (Ewald et al., 2010). TIS may be a double-edged sword; on the upside, cellular senescence constitutes a barrier against malignant progression; and on the downside, the long-term persistence of senescent cancer cells carries liabilities associated with their proinflammatory secretory phenotype (Coppé et al., 2010) and risks such as their resistance to apoptosis, and their potential for re-entering the cell cycle (Roberson et al., 2005). Melanomas arising from nevi are good examples for escape of oncogene-driven cells from senescence (Dhomen et al., 2009). A related paradigm is slow-cycling stem-like cancer cells. Like TIS cells, slow-cycling cells are drug-resistant (Dean et al., 2005) and can therefore be selected with various cytotoxic agents (as described in Roesch et al., 2013 and sections below). Reactivation of therapy-selected quiescent tumor cells might therefore contribute to a significant portion of relapses after therapy.

Eradicating cancer cells escaping drug-induced apoptosis by entering a TSQ state would therefore appear an important goal in cancer therapy. This ambition hardly meshes with our limited knowledge of specific vulnerabilities of quiescent/slow-cycling tumor cells that could be targeted for therapy. Several recent reports have presented data that strongly suggest mitochondrial respiration as a specific vulnerability of TSQ cells, which when exploited should be synthetically lethal with established cytotoxic therapies (Corazao-Rozas et al., 2013; Dörr et al., 2013; Haq et al., 2013; Roesch et al., 2013).

Increased Mitochondrial Activity in Quiescent/Slow-Cycling Cancer Cells

As originally discovered by Otto Warburg, rapidly proliferating cancer cells are typically marked by high rates of aerobic glycolysis, a metabolic change that is exploited today in positron emission tomography (PET) imaging of tumors. This property is a universal hallmark shared by all rapidly dividing eukaryotic cells including single celled yeasts. Quiescent cells, in contrast, appear to rely on complete oxidation of pyruvate to CO_2 to meet their ATP demands. Aerobic glycolysis, in addition to other metabolic changes, may serve to optimally support the anabolic requirements of proliferating cancer cells (Lunt and Vander Heiden, 2011; Ward and Thompson, 2012). It enables cancer cells to divert glucose carbon from mitochondrial ATP production to the synthesis of biomolecules needed for biomass accumulation and cell division. In the simplest scenario, one might predict that the metabolism of TSQ cells resembles that of resting normal cells (i.e., high rate of mitochondrial respiration) rather than that of the proliferating tumor cells from which they were selected (anaerobic glycolysis). Recent studies have shown that this is true, at least for some TSQ cells.

Lymphoma cells undergoing TIS were found to be metabolically hyperactive, a remarkable finding in light of their nonproliferative state (Dörr et al., 2013). Eμ-myc lymphoma cells

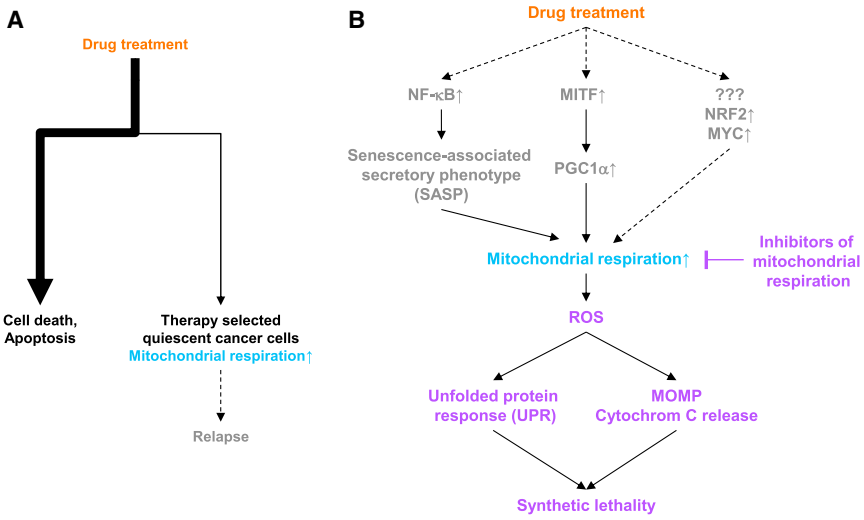

Figure 1. Models of Therapy-Induced Quiescence Leading to Upregulation of Mitochondrial Respiration and Effect of Mitochondrial Inhibitors

(A) Drug treatment leads to apoptotic cell death in the bulk of the tumor mass. A minority of cells escape into a quiescent/senescent state with upregulated OXPHOS. When these cancer memory cells escape quiescence, they give rise to relapse.

(B) Based on the reports summarized in this article, drug treatment would select for slow-cycling therapy-resistant cancer cells in which transcriptional pathways are upregulated that induce ETC proteins and OXPHOS. OXPHOS upregulation is expected to selectively sensitize drug selected cells to mitochondrial inhibitors (depicted in purple), resulting in ROS formation and downstream events that cumulate in synthetic lethality of the drug-resistant cancer.

rendered incompetent for apoptosis by overexpression of BCL2, responded to treatment with cyclophosphamide or adriamycin by uniformly undergoing TIS as indicated by decreased BrdU incorporation and positivity for senescence-associated β-galactosidase (Dörr et al., 2013). Consistent with their TIS phenotype, these tumors are quiescent in vivo as shown by undetectable signals in [^{18}F]fluorothymidine PET. Unexpectedly, however, these drug-treated TIS tumors exhibit a stronger signal than untreated tumors in [^{18}F]fluorodeoxyglucose PET, indicating that they have increased glucose uptake. In vitro, the Eμ-myc;BCL2 lymphoma cells also show increased glucose uptake and metabolism to pyruvate and lactate. At first sight, it thus appears that TIS reinforces the Warburg effect.

However, Eμ-myc;BCL2 lymphoma cells undergoing TIS also showed several signs untypical of the Warburg effect: (1) they not only accumulated lactate, but also citrate, indicating that TIS cells also increased flux into the tricarboxylic acid (TCA) cycle; (2) they upregulated isoform 1 of pyruvate kinase (PKM1), thus providing abundant fuel for the TCA cycle; and (3) they activated AMP-activated protein kinase (AMPK) signaling, suggesting that their energy demand is greater than what can be met through anaerobic glycolysis. All three findings indicated that unlike with proliferating cancer cells, increased glucose utilization of TIS cells drives the TCA cycle that feeds into mitochondrial respiration. Consistent with this interpretation are the observations that mitochondrial oxygen consumption rate and intracellular ATP levels were strongly increased in Eμ-myc;BCL2 lymphoma cells undergoing TIS. These cells display a hybrid form of energy metabolism, marked by high levels of glucose utilization, mitochondrial respiration, and fatty acid oxidation, apparently geared toward maximizing ATP production.

Similar upregulation of mitochondrial respiration was found in drug selected slow-cycling cancer cells, which are required for continuous maintenance of melanomas (Roesch et al., 2010). JARID1B/KDM5B is a histone 3 K4 demethylase that is highly expressed in benign nevi, which undergo oncogene-induced senescence (Dhomen et al., 2009); but it is downregulated in primary and metastatic melanomas (Roesch et al., 2005). Due to tumor heterogeneity, a small portion of melanoma cells typically retains JARID1B expression, however. Even subpopulations of cultured melanoma cell lines switch to a JARID1B^{high} phenotype in an apparently stochastic fashion (Gupta et al., 2011; Roesch et al., 2010). These JARID1B^{high} cells cycle very slowly, but have increased self-renewal capacity, stem cell-like properties, and are required for melanoma maintenance in vitro and in vivo (Roesch et al., 2010). Like TIS cells, slow-cycling JARID1B^{high} cells are drug-resistant and can therefore be selected with various cytotoxic agents (cisplatin, bortezomib, vemurafenib, temozolomide, etc.) (Roesch et al., 2013). Proteomic profiling of these cells revealed a striking upregulation of proteins involved in mitochondrial respiration (OXPHOS), including subunits of complexes I, III, IV, and ATP synthase (Roesch et al., 2013). This resulted in increased oxygen consumption, increased mitochondrial ATP levels, and increased production of H_2O_2.

In summary, an unexpected common denominator of these scenarios of therapy-selected quiescence is metabolic reprogramming to upregulate OXPHOS genes and increase oxygen consumption and ATP production (Dörr et al., 2013; Roesch et al., 2013).

Increased Energy Demand of Cancer Cells Undergoing Therapy-Induced Senescence

Why is the energy demand of seemingly quiescent cancer cells so high that it cannot be met without upregulating mitochondrial activity? It turns out that the TIS cells studied by Dörr et al. (2013) have ramped up three highly energy consuming pathways: protein synthesis and folding, protein ubiquitylation, and proteasomal degradation.

Various forms of senescence are known to coincide with the so-called senescence-associated secretory phenotype (SASP), a NFκB driven program to synthesize copious amounts of secretory proteins (Chien et al., 2011; Coppé et al., 2010; Freund et al., 2011). Increased protein synthesis and flux through the secretory pathway put high demands on the protein folding capacity of chaperones in the ER. When this capacity is exhausted, ER stress will result, which leads to induction of the unfolded protein response (UPR) (Cao and Kaufman, 2012; Walter and Ron, 2011). Eμ-myc;BCL2 lymphoma cells undergoing TIS show a strong induction of the UPR. UPR signaling sets into motion a program that directs either adaptation or cell death (Kim et al.,

2008; Tabas and Ron, 2011). The adaptive response is geared toward increasing the ER protein folding capacity through induction of chaperones and the removal of unfolded proteins by upregulating ER-associated degradation through the proteasome and autophagy pathways. The apoptotic response, which may be a consequence of sustained, irreparable ER stress, involves several mitochondria-dependent and independent pathways (Kim et al., 2008; Tabas and Ron, 2011). Of particular importance are the UPR-induced transcription factors ATF4 and CHOP, which stimulate protein synthesis, causing ATP depletion, oxidative stress, and apoptotic cell death (Han et al., 2013).

Despite UPR activation and strong induction of ATF4 and CHOP, Eμ-myc:BCL2 lymphoma cells in TIS do not undergo apoptosis. This could be due to a combination of factors such as overexpression of BCL2, induction of NFκB (as part of SASP), and maintenance of high ATP levels due to upregulation of glycolysis and OXPHOS. Instead of inducing apoptosis, UPR signaling may reinforce or even drive the quiescent state of TIS cells in the absence of functional p53 and RB1 tumor suppressor pathways. For example, the UPR is known to suppress cyclin D1 synthesis via phosphorylation of eIF2α (Brewer et al., 1999). Nevertheless, it is likely that TIS cells live on the edge with respect to proapoptotic signaling.

Whether the increased OXPHOS occurring in drug-resistant slow-cycling stem-like cancer cells (Roesch et al., 2013) sustains a similar anabolic secretory program as found in TIS cells is currently unknown, as is the status of UPR signaling in these cells. It is noteworthy, however, that just like TIS cells, stem-like cancer cells are known to display increased NFκB activity, which could potentially drive a secretory program (Fulda, 2013).

How Is Mitochondrial Respiration Upregulated in Response to Therapy?

Yet another recent example of drug-induced OXPHOS, again in melanoma, has shed light on this important question. Upregulation of OXPHOS genes and an increase in mitochondrial biogenesis and ATP levels was found to coincide with BRAF(V600E) mutant melanomas acquiring resistance to BRAF inhibitors (Haq et al., 2013). OXPHOS upregulation was found to occur via microphthalmia-associated transcription factor (MITF), a melanocyte-lineage transcription factor, and bona fide melanoma oncogene (Garraway et al., 2005). MITF directly drives the expression of proliferator-activated receptor γ (PPARγ) coactivator-1α (PGC1α and PPARGC1A) (Figure 1B, a known master regulator of mitochondrial biogenesis, see Scarpulla et al., 2012). In fact, 10% of human melanomas overexpress MITF, PGC1α, and OXPHOS genes and have increased respiration even without BRAF inhibitor treatment (Vazquez et al., 2013). Genetic evidence demonstrates that such tumors depend on PGC1α for survival and tumor progression. In addition, MITF/PGC1α-dependent OXPHOS upregulation confers resistance to cancer drugs whose mechanism-of-action involves apoptosis induced by reactive oxygen species (ROS) (Vazquez et al., 2013).

It is tempting to speculate that TIS and slow-cycling stem-like cancer cells also invoke PGC1α induction to upregulate OXPHOS. For example, Eμ-myc lymphoma cells undergoing TIS show phosphorylation of adenosine monophosphate AMPK (Dörr et al., 2013), a known activator of PGC1α (Scarpulla et al., 2012). However, not all melanomas resistant to BRAF inhibition upregulate OXPHOS via PGC1α (Corazao-Rozas et al., 2013). The remaining tumors may utilize some of the other transcriptional pathways known to regulate mitochondrial biogenesis, for example Nuclear Respiratory Factor 1, (NRF)2, and/or MYC (Scarpulla et al., 2012). It is also noteworthy that the small fraction of melanomas that are intrinsically resistant to BRAF inhibition (as opposed to acquiring resistance in response to BRAF inhibition) are marked by low, rather than high, MITF expression (Konieczkowski et al., 2014). Overall, the MITF-PGC1α interplay is complex due to mutual feedforward regulation (Ronai, 2013).

Reliance on Mitochondrial Respiration as a Cancer Selective Vulnerability

Exploiting the altered metabolism of cancer cells for therapy has become a widely accepted concept (Schulze and Harris, 2012). The studies discussed here strongly suggest that reliance on OXPHOS may represent a previously unexpected "druggable" vulnerability of (multi)drug-resistant cancers. Minimally, this would apply to lymphomas undergoing TIS in response to cytotoxic therapy (Dörr et al., 2013), to slow-cycling melanoma cells selected for a JARID1B^{high} phenotype by cytotoxic agents (Roesch et al., 2013), and to BRAF mutant melanomas escaping therapy with BRAF inhibitors (Haq et al., 2013). These examples make a strong case for inhibitors of OXPHOS to combat drug-resistant cancer, especially melanomas and lymphomas.

Experimental support for this proposition is provided by the antitumor activity of genetic ablation of OXPHOS components in melanomas (Vazquez et al., 2013). In addition, various chemical inhibitors of OXPHOS were able to overcome resistance to widely used targeted and cytotoxic treatments (Table 1). Thus, mitochondrial inhibitors proved effective for synthetic lethal targeting of drug-resistant cancer cells marked by high OXPHOS activity.

Whereas most of the mitochondrial inhibitors and decouplers employed in the above studies have narrow therapeutic indices and are for laboratory use only, there is a sizeable collection of clinically used drugs that inhibit the electron transport chain (ETC). These include, for example, tamoxifen and metformin, which inhibit complex I; resveratrol, a complex III antagonist; and the complex V inhibitor 3,3-diindolylmethane (reviewed in Rohlena et al., 2011; Toogood, 2008). Metformin, in particular, was found highly effective against stem-like cancer cells and to synergize with chemotherapeutics in the mouse (Hirsch et al., 2009; Iliopoulos et al., 2011). In addition, there are a number of emerging mitochondrial inhibitors that have been successfully used in rodent tumor studies with manageable toxicity, at least in the short term (Corazao-Rozas et al., 2013; Rico-Bautista et al., 2013; Rohlena et al., 2011). These substances may merit future development into clinical candidates.

Mechanism-of-Action of Mitochondrial Inhibitors as Anticancer Agents

ATP levels may not be limiting for cancer cells (Scholnick et al., 1973). To the contrary, some cancer cells engage various modes of dissipating ATP levels in order to alleviate negative feedback inhibition on glycolysis, a mechanism required for efficient flux of glucose carbon into macromolecular synthesis pathways (Fang et al., 2010; Vander Heiden et al., 2010). It is therefore unlikely that the cytotoxicity of OXPHOS inhibitors in the described

Table 1. Synthetic Lethal Combinations of Mitochondrial Inhibitors with Established Cancer Treatments

Reference	Cancer	Mitochondrial Inhibitor	Target	Synthetically Lethal with
Roesch et al., 2013	melanoma	rotenone	complex I (NADH:ubiquinone oxidoreductase)	cisplatin, in vitro
		oligomycin	complex V (F0/F1 ATPase)	cisplatin, in vitro
		phenformin	complex I	vemurafenib, in vivo
Haq et al., 2013	melanoma	rotenone	complex I	vemurafenib, in vivo
		TTFA (thenoyltrifluoroacetone)	complex II (Succinate dehydrogenase)	vemurafenib, in vivo
		oligomycin	complex V (F0/F1 ATPase)	vemurafenib, in vivo
		2,4-dinitrophenol	mitochondrial uncoupler	vemurafenib, in vivo
		CCCP (carbonyl cyanide m-chlorophenyl hydrazone)	mitochondrial uncoupler	vemurafenib, in vivo
Dörr et al., 2013	lymphoma	antimycin A	complex III (ubiquinone-cytochrome c oxidoreductase)	adriamycin, in vitro

drug-resistant settings arises from ATP depletion. Rather, cell death appears to result from the deleterious effects of ROS. Indeed, the proapoptotic activity of mitochondrial inhibitors can be reversed by antioxidants (as described in Rico-Bautista et al., 2013; Sundberg et al., 2009; Watabe and Nakaki, 2007), suggesting that oxidative stress rather than ATP depletion is the primary trigger of apoptosis in response to ETC inhibition. At the same time, inhibition of ROS detoxifying systems such as superoxide dismutase (SOD), glutathione, or thioredoxins would seem to bear potential in combination with mitochondrial inhibitors. Several inhibitors of ROS detoxification are under development (Wondrak, 2009).

With regards to mechanism-of-action, it is well established that inhibition of ETC complexes causes major leakage of electrons and subsequent superoxide and hydrogen peroxide generation (reviewed in Rohlena et al., 2011). This is especially true for cancer cells that have upregulated OXPHOS (Corazao-Rozas et al., 2013; Vazquez et al., 2013). Although small increases in ROS can be protumorigenic due to damage to macromolecules, high levels of ROS induce apoptosis through various mechanisms, including permeabilization of the outer mitochondrial membrane and cytochrome C release (Madesh and Hajnóczky, 2001), as well as induction of the UPR (Rico-Bautista et al., 2013) (Figure 1B), which is known to engage in a self-reinforcing cycle with oxidative stress (Malhotra and Kaufman, 2007). This "threshold concept" of ROS activity and the preferential sensitivity of cancer cells toward apoptosis induced by high levels of ROS is now widely established and provides a solid basis for cancer selectivity of mitochondrial inhibitors (reviewed in Kong et al., 2000; Luo et al., 2009; Trachootham et al., 2009; Watson, 2013; Wondrak, 2009).

Which Cancer Patients Might Benefit from Mitochondrial Inhibitors?

If these concepts are well established in the basic science literature, why have mitochondrial inhibitors been slow in coming in the clinic? A reason may be that it has been challenging to predict which patients would benefit from such therapies. Several recent clinical trials failed to show benefits. For example, the mitochondrial inhibitor elesclomol in combination with paclitaxel did not increase progression-free survival in a phase III melanoma trial (O'Day et al., 2013). Likewise, retrospective studies of the effects of metformin, at antidiabetic doses, on prostate cancer mortality have delivered contradictory results that dampened overall enthusiasm (Azvolinsky, 2014). Although the effectiveness of metformin is more appropriately tested in randomized prospective trials, which are now underway, even those may fail if performed in an unselected patient cohort. Notably, the "flopped" elesclomol trial actually did reveal a statistically significant improvement in median progression-free survival for the elesclomol/paclitaxel combination in a subgroup of patients with normal, as opposed to increased, levels of lactate dehydrogenase (O'Day et al., 2013). High levels of this metabolic enzyme, which catalyzes the conversion of pyruvate to lactate, may stimulate anaerobic glycolysis, thereby enabling escape from mitochondrial inhibition.

Considering the points above, clinical testing of mitochondrial inhibitors stands to win as much from effective biomarkers as any other form of new therapy. A main significance of the new studies summarized in this article (Corazao-Rozas et al., 2013; Dörr et al., 2013; Haq et al., 2013; Roesch et al., 2013; Vazquez et al., 2013) may lie with their opening of a treasure trove of potential biomarkers for patient stratification in future clinical trials (Table 2). Chief among these would be OXPHOS components, such as complex I–V subunits and oxidative stress defenders such as hemeoxigenase 1, SOD, and catalase (CAT) genes, which were repeatedly found upregulated in drug-resistant cancers. Upstream regulators of OXPHOS gene expression such as PCG1α and NRF2 would also seem promising candidate biomarkers. While nuclear accumulation of NRF2 mediates an adaptive response to oxidative stress and is thus typically thought of as an indicator of chemoresistance (Villeneuve et al., 2010), recent evidence implicated the transcription factor in a threshold-dependent feedforward loop of ROS generation cumulating in cell death (Zucker et al., 2014). In addition, prostate cancer cells with constitutively nuclear NRF2 are exquisitely sensitive to the mitochondrial inhibitor SMIP004 (Rico-Bautista et al., 2013). It is thus possible that high levels of nuclear NRF2 in advanced cancers correspond with sensitivity to inhibitors of OXPHOS, although more detailed validation is required. AMPK may also be a surrogate marker for energy stress in drug-resistant cancer with increased mitochondrial activity (Dörr et al., 2013). More classical indicators of redox and energy status, such as the levels of reduced glutathione, ROS, and ATP, as

Table 2. Potential Biomarkers to Identify Patients Benefitting from Mitochondrial Inhibitors

Type	Potential Biomarkers	Cancer Type Tested
Dörr et al., 2013		
Proteins	phosphorylated AMPK (phospho-AMPK)-T172	up in lymphoma cells undergoing TIS
	M1 isoform of pyruvate kinase (PKM1)	up in lymphoma cells undergoing TIS
	activating transcription factor 4 (ATF4)	up in lymphoma cells undergoing TIS
	C/EBP-Homologous Protein (CHOP)	up in lymphoma cells undergoing TIS
	phosphorylated JUN N-terminal kinase (phospho-JNK)-T183/185	up in lymphoma cells undergoing TIS
	high molecular weight ubiquitin conjugates	up in lymphoma cells undergoing TIS
	sequestosome 1 (SQSTM1/p62)	up in lymphoma cells undergoing TIS
	microtubule-associated protein 1 light chain 3 (MAP1-LC3) isoform II	up in lymphoma cells undergoing TIS
	lysosomal vacuolar type ATPase (V-ATPase A1)	up in lymphoma cells undergoing TIS
Metabolites	citrate/glucose ratio	up in lymphoma cells undergoing TIS
	lactate/glucose ratio	up in lymphoma cells undergoing TIS
	pyruvate/glucose ratio	up in lymphoma cells undergoing TIS
	ATP	up in lymphoma cells undergoing TIS
Haq et al., 2013		
Organelle	mitoTracker Green/Red (mitochondrial density)	up in BRAF inhibitor treated melanoma cells
Proteins	MITF	up in BRAF inhibitor treated melanoma cells
	peroxisome proliferator-activated receptor g coactivator 1 (PPARGC1A/PGC-1α)	up in BRAF inhibitor treated melanoma cells
mRNAs	MITF	up in BRAF inhibitor treated melanoma cells
	peroxisome proliferator-activated receptor γ coactivator 1 (PPARGC1A/PGC-1α)	up in BRAF inhibitor treated melanoma cells
	succinate dehydrogenase (complex II) subunit B (SDHB)	up in BRAF inhibitor treated melanoma cells
	succinate dehydrogenase (complex II) subunit D (SDHBSDHD)	up in BRAF inhibitor treated melanoma cells
	mitochondrial F1 ATP synthase (complex V) beta subunit (ATP5B)	up in BRAF inhibitor treated melanoma cells
	mitochondrial F1 ATP synthase (complex V) delta subunit (ATP5D)	up in BRAF inhibitor treated melanoma cells
	mitochondrial F0 ATP synthase (complex V) subunit 9 (ATP5G1)	up in BRAF inhibitor treated melanoma cells
	cytochrome C oxidase (complex IV) subunit 15 (COX15)	up in BRAF inhibitor treated melanoma cells
	CAT	up in BRAF inhibitor treated melanoma cells
	superoxide dismutase 2 (mitochondrial) (SOD2)	up in BRAF inhibitor treated melanoma cells
Metabolite	mitochondrial ROS (MitoSOX)	up in BRAF inhibitor treated melanoma cells
Roesch et al., 2013		
Proteins	jumonji/ARID domain-containing demethylase (JARID1B)	up in therapy selected melanoma cells
	NADH:ubiquinone oxidoreductase (complex I) alpha subcomplex subunit 4 (NDUFA4)	up in therapy selected melanoma cells
	NADH:ubiquinone oxidoreductase (complex I) beta subcomplex subunit 1 (NDUFB1)	up in therapy selected melanoma cells
	ubiquinone-cytochrome c oxidoreductase (complex III) subunit 7 (UQCRB)	up in therapy selected melanoma cells
	cytochrome C oxidase (complex IV) subunit 7A2 (COX7A2)	up in therapy selected melanoma cells
	mitochondrial F0 ATP synthase (complex V) B chain (ATP5F1)	up in therapy selected melanoma cells
	mitochondrial F0 ATP synthase (complex V) subunit g (mitochondrial ATP5L)	up in therapy selected melanoma cells
	hexokinase 1 (HK1)	down in therapy selected melanoma cells

(*Continued on next page*)

Table 2. *Continued*

Type	Potential Biomarkers	Cancer Type Tested
	Hexokinas 2 (HK2)	down in therapy selected melanoma cells
Corazao-Rozas et al., 2013		
Protein	CAT	up in BRAF inhibitor treated melanoma cells
Metabolites	ratio of oxidized/reduced glutathione (GSSG/GSH)	up in BRAF inhibitor treated melanoma cells
Vazquez et al., 2013		
mRNAs	peroxisome proliferator-activated receptor γ coactivator 1 (PPARGC1A/PGC-1α)	up in PGC1α overexpressing melanoma cells
	ERRα	up in PGC1α overexpressing melanoma cells
	isocitrate dehydrogenase 3 (NAD+) alpha (IDH3A)	up in PGC1α overexpressing melanoma cells
	NADH:ubiquinone oxidoreductase (complex I) 30 kDa subunit (NDUFS3)	up in PGC1α overexpressing melanoma cells
	cytochrome C oxidase (complex IV) subunit Va (COX5A)	up in PGC1α overexpressing melanoma cells
	mitochondrial F0 ATP synthase (complex V) subunit 9 (ATP5G1)	up in PGC1α overexpressing melanoma cells
	cytochrome C (CYTC)	up in PGC1α overexpressing melanoma cells
Proteins	peroxisome proliferator-activated receptor g coactivator 1 (PPARGC1A/PGC-1α)	up in PGC1α overexpressing melanoma cells
	mitochondrially encoded cytochrome C oxidase I (MT-CO1)	up in PGC1α overexpressing melanoma cells
	mitochondrial F1 ATP synthase alpha subunit (ATP5A1)	up in PGC1α overexpressing melanoma cells
	ubiquinone-cytochrome c oxidoreductase (complex III) core subunit 2 (UQCRC2)	up in PGC1α overexpressing melanoma cells
Metabolites	Citrate	down in melanoma cells engineered to downregulate PGC1α
	Aconitate	down in melanoma cells engineered to downregulate PGC1α
	Isocitrate	down in melanoma cells engineered to downregulate PGC1α
	Fumarate	down in melanoma cells engineered to downregulate PGC1α
	Malate	down in melanoma cells engineered to downregulate PGC1α
	ATP	down in melanoma cells engineered to downregulate PGC1α
	Lactate	up in melanoma cells engineered to downregulate PGC1α
	dihydroxyacetone phosphate, DHAP	up in melanoma cells engineered to downregulate PGC1α
	ATP	up in melanoma cells engineered to overexpress PGC1α
	Lactate	down in melanoma cells engineered to overexpress PGC1α

well as oxygen consumption might also be considered, although these are less practical to measure, at least in fixed clinical material.

It is anticipated that mitochondrial inhibitors could be used either as second-line treatment of drug-resistant cancers or even as first-line treatment in combination with standard-of-care cytotoxic or targeted therapies (Figure 1B). Biomarker-guided clinical trials will be necessary to rigorously assess these options.

Potential Limitations

Despite these promises, some important unknowns remain that will require further research to be resolved. For example, it is too early to say how pervasive the switch to oxidative metabolism in TSQ cells is. Is it limited to the lymphoma and melanoma models discussed here, or is it a general characteristic of quiescent cells as has been proposed (reviewed in Lunt and Vander Heiden, 2011; Ward and Thompson, 2012)? Once again, molecular profiling—e.g., for the signatures outlined in Table 2—of other cancer models and patient samples may assist in answering this question. The important factor here is not necessarily the actual fraction of drug-resistant cancers that show the respiration phenotype, but rather the sensitivity and specificity with which biomarkers can identify this fraction.

Likewise, the concept emerging from the observations summarized here proposing that disruption of OXPHOS affords a level of selectivity for cancer cells that is required for a successful therapeutic approach has not yet been conclusively established.

This uncertainty is especially worrisome for normal cells with a high demand of mitochondrial ATP such as cardiomyocytes or with limited ability to withstand oxidative stress (cardiomyocytes, dopaminergic neurons, etc.). Further research is required to assess whether the selective upregulation of OXPHOS, observed in drug-resistant cancers, creates enough of a therapeutic window to effectively and safely intervene with mitochondrial inhibitors. This being said, it should not be forgotten that several mitochondrial inhibitors have been in safe clinical use for decades. It is also worth noting that the toxicity associated with mitochondrial inhibitors can be off-target effects. For example, the neurotoxicity of rotenone and methyl-4-phenylpyridinium is not a result of their activity in inhibiting complex I, but rather to their off-target activity of disrupting microtubules (Choi et al., 2011). Piericidin, another complex I inhibitor that does not disrupt microtubules, is not neurotoxic. This example illustrates that neurotoxicity is not an obligatory adverse effect of mitochondrial inhibitors.

As noted above, a main issue with agents such as metformin and elesclomol in clinic trials has not been toxicity, but rather lack of efficacy. These failures may indicate either ineffective selection of patients most likely to benefit from mitochondrial inhibitors based on biomarkers or rapid development of resistance to this therapeutic approach. It has been established for decades that eukaryotic cells can be adapted to ρ^0 cells, which lack mitochondrial DNA, demonstrating that respiration activity is not strictly essential for survival, at least under nutrient rich conditions. In addition, a recent study demonstrated that increased glycolysis could compensate for inhibition of OXPHOS affected by downregulating PGC1α in melanoma cells (Lim et al., 2014). The escape was due to ROS-mediated stabilization of HIF1α, which in turn upregulated glycolytic genes. Although it remains to be seen whether pharmacological inhibition of OXPHOS would lead to a similar adaptation, these findings suggest that, as with many other cancer therapies, the real potential of mitochondrial inhibitors may lie with combining them with inhibitors of other metabolic pathways such as glycolysis, glutamine utilization, or ROS detoxification.

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